THE DEVELOPMENT AND EVALUATION OF A MULTIPLEX REAL TIME PCR ASSAY FOR THE DIAGNOSIS OF *ACANTHAMOEBA* AND HERPES SIMPLEX KERATITIS

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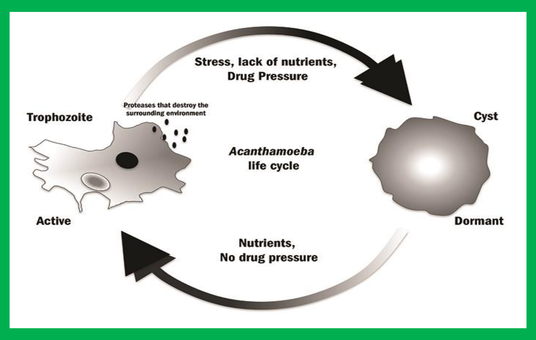
Introduction

The inflammation of the cornea caused by Acanthamoeba and herpex simples virus is called *Acanthamoeba* keratitis (AK) and herpatic keratitis (HR) respectively. The most common symptoms of keratitis are eye redness, pain, blurred vision, photophobia, irritation and excess tearing.



(www.opticianonline.net)

Acanthamoeba is an *opportunistic protozoa* present in a variety of habitats as an active trophozoite or dormant cyst form. *More cases of AK are reported in contact lens users (85-88%).* HSV - 1 is a more common cause of ocular infection and can cause four types of keratitis. Accurate, fast diagnosis and treatment of AK and HK are essential to *prevent the permanent damage of cornea and ultimately loss of sight.* Current diagnostic approaches are time-consuming, lack sensitivity, **expensive and require further interventions.**

Aims: *Develop a Multiplex PCR assay that can detect AK and HK ensuring good quality of sample and authenticity of the process.*

Keyword; Acanthamoeba, Herpetic, Keratitis, Multiplex , Assay, PCR, specimen, diagnosis.

Methods

- Two sets of Acanthamoeba P+P used from previous journals.
- HSV P+P used from in-houseTaqman PCR assay.
- *A. castellanii* and AcroMetrix Multi-Analyte used as +ve controls.
- RNaseP used to determine corneal sample adequacy.
- DNA IC for the extraction process.
- The assays optimised and multiplexed together on LC PCR.
- 8 strains of *Acanthamoeba* cultured on Page's agar plates.
- KOVA glasstic slide used to count the cysts.
- NA extracted on Roche MagNaPure.
- Amplification was done on LightCycler PCR.
- Comparison between LC and Taqman with 26 HSV strong and weak positive samples.
- 14 HSV clinical specimens spiked with *Acanthamoeba +ve control.*
- The reproducibility test perfomed on 5 serial dilution of *Acanthameoba* and HSV positive controls run in replicates of 5 per run, performed on four days.
- *50 corneal scrapes* and *60 eye swabs* tested to determine SENSITIVITY and SPECIFICITY of the assay.

Results

- *Acanthamoeba LOD* = ***1 copy of genome/ reaction***
- HSV LOD = **1 genome in 10000 dilutions**
- On Lightcycle, HSV Ct range = 18.74 – 37.10
- On ABI Taqman HSV Ct range = 18.86 - 44.24.
- Efficiency of Multiplex PCR assay = 98% -100%
- *Cell count = A. polyphaga* max, clinical specimen mini
- Duplex PCR detects1/1000000 dilution of *A. strains*
- Monoplex PCR assay detects 1/100000 dilution
- *No detection of A. astronyx.*
- Reproduciblity CoV of 0.822 and 0.602 .

Sensitivity = 100%, Specificity = 98% and 100%

Assay	No of samples	PCR positive	Sensitivity (%) (95 % CI)	Specificity (%) (95 % CI)
Acanthamoeba	50	3	100 20% to 100%	98 88% to 100%
HSV	60	18	100 78% to 100%	100 90% to 100%

Discussion

During early stages *Acanthamoeba* keratitis usually represents with mimic herpetic epithelial keratitis. This can lead to either missed or delayed diagnosis of keratitis that can cause severe ocular damage. Therefore, *the purpose of this study was to develop a multiplex PCR assay that can diagnose AK and HK simultaneously.*

The corneal scrapping sample is the preferred sample for reliable diagnosis of AK. This is because *Acanthamoeba* penetrates deep into cornea, therefore superficial swabs or tears are often unsuitable for AK detection in advance stages of infection or if pre-treated with antibiotics.

The set of all primers and probes used in *this assay run at a 60°C elongation temperature*, therefore, the assay can be loaded on a single PCR plate in one well to achieve high sensitivity and short turnaround time.

There are some limitations, such as, the new assay was unable to detect *Acanthamoeba astronyx*, a small number of positive *Acanthamoeba* were tested and the assay contains HSV probe 1 & 2 with same flourescence dye. Therefore, this project could be improved by using two separate fluorescence dyes for HSV probe 1 and 2.
Once the assay is put into a routine use by Roche flow, it is expected that majority of the samples would be tested on the same day to get *results within 24 hours*.

This assay improves the turnaround time for keratitis diagnosis and reporting of the results to benefit patient management with use of appropriate and targeted antibiotic treatment.

Conclusions

- This Multiplex PCR assay provides better sensitivity and specificity to both *Acanthamoeba* and HSV, also ensure the sample quality and extraction process.
- However, one culture positive *Acanthamoeba* strain *(A. Astronyx)* was not detected by this Multiplex PCR assay.
- Therefore, culturing of *Acanthamoeba specimen* cannot be discontinued, but will be augmented by PCR.
- This assay is a great success to provide fast and accurate diagnosis of *Acanthamoeba* and herpetic keratitis.

References

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Acknowledgement

I'm thankful to Public Health England (PHE), Manchseter Eye Hospital, IBMS and the University of Greenwich, London.